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Lipopolysaccharide decreases bradykinin receptor-induced acidification responses in cultured bovine aortic endothelial cells

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Abstract

The effects of bacterial lipopolysaccharide (*Escherichia coli* 0127-B8) on bradykinin receptor function in bovine aortic endothelial cells were investigated using a microphysiometer. Bradykinin and Lys^0–desArg^{10}-bradykinin produced concentration-dependent acidification responses with pEC_{50} values of 8.87 ± 0.20 and 9.78 ± 0.08, respectively. These responses were competitively and selectively antagonised by the bradykinin B_2 receptor antagonist, icatibant and the bradykinin B_1 receptor antagonist, desArg^9–Leu^8-bradykinin, respectively. The non-peptide bradykinin B_2 receptor antagonist, FR173657 (0.3 and 3 nM), selectively antagonised bradykinin-induced acidification responses, causing rightward shifts of the concentration–response curves to bradykinin, but at the same time, significantly decreasing the maximum response. A preincubation with lipopolysaccharide (0.01 and 0.1 μg/ml) for 24 h caused a significant concentration-dependent decrease in maximal response to bradykinin (27.2 ± 1.9 and 9.7 ± 0.4% of control) and the bradykinin B_1 receptor agonist, Lys^0–desArg^{10}-bradykinin (59.0 ± 7.14 and 25.3 ± 7.8% of control), without affecting the EC_{50}. These results suggest that bradykinin B_1 receptors are constitutively expressed in cultured bovine aortic endothelial cells and that the microphysiometer provides a rapid, sensitive technique to characterise bradykinin receptors and investigate their regulation by cytokines. Interactions between bradykinin receptors and lipopolysaccharide may play a part in the cascade of deleterious effects that occur during septic shock.

Keywords: Lipopolysaccharide, bacterial; Bradykinin receptor; Microphysiometer; Aortic endothelial cell, bovine; FR173657

1. Introduction

The kinins, a group of peptides the best known of which is bradykinin, are synthesised at the sites of tissue damage by the enzymatic action of tissue and plasma kallikrein on large protein precursors, the kininogens (Bhoola et al., 1992). In addition to their well-known inflammatory role, kinins are also involved in the regulation of vascular tone. Kinins exert the majority of their effects by the interaction with cell surface receptors which were originally classified into two subtypes, B_1 and B_2, according to the relative potency of naturally occurring peptide agonists (Regoli and Barabé, 1980) and, more recently, the existence of these two subtypes has been confirmed by the development of selective bradykinin receptor antagonists as well as by molecular biology techniques (see Hall, 1997). Bradykinin and kallidin are potent agonists for the bradykinin B_2 receptor whereas further cleavage of these endogenous agonists into desArg^9-bradykinin or desArg^{10}-kallidin, for example, is required for the activation of bradykinin B_1 receptors (Regoli and Barabé, 1980). Icatibant (Hoe-140) is a potent selective antagonist of the bradykinin B_2 receptor (Hock et al., 1991) and although extremely useful for the classification of bradykinin receptors, its therapeutic use is limited due to its peptide nature and, hence, its poor oral availability. Recently, an orally active, highly potent non-peptide bradykinin B_2 receptor antagonist, FR173657, has been described (Asano et al., 1997). Peptide antagonists are available for the classification of bradykinin receptors into the B_1 subtype, the most commonly used of which is desArg^9–Leu^8-bradykinin (Regoli et al., 1977).

Bradykinin B_2 receptors are constitutively expressed in many cell types and the majority of the actions of kinins

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are mediated by this receptor (see for reviews, Regoli et al., 1990; Dray and Perkins, 1993). Usually, under normal physiological conditions, bradykinin B_1 receptor-mediated responses are absent but are induced following tissue trauma. For example, induction of bradykinin B_1 receptor-mediated responses occurs during in vitro incubation of tissue (Regoli et al., 1977; Bouthillier et al., 1987; Deblois and Marceau, 1987), or after treatment with bacterial lipopolysaccharide (Barabé et al., 1982; Galizzi et al., 1994), or interleukin-1β (Deblois et al., 1988, 1991). In addition, numerous in vivo studies have described the induction of a state of responsiveness to bradykinin B_1 receptor agonists by treatment with lipopolysaccharide or interleukin-1β (Marceau et al., 1984; Drapeau et al., 1991; Davis and Perkins, 1994; Perkins et al., 1995). Due to the inducible nature of bradykinin B_1 receptors, it has been suggested that bradykinin B_2 receptors are responsible for the early stages, and bradykinin B_1 receptors are responsible for the maintenance, of inflammatory processes (Galizzi et al., 1994). Thus, it is possible, at least in part, that the induction of bradykinin B_1 receptors and, hence, modulation of nitric oxide (NO) activity is the underlying cause of the massive vasodilatation and hypotension which develops during septic shock, a disease in which lipopolysaccharide, a protein on the outer surface of bacteria, causes the release of inflammatory mediators, including cytokines (Beutler and Cerami, 1986).

The presence of the endothelium appears to be important in the vasorelaxation response of isolated vessels to desArg^9-bradykinin (DeBlois and Marceau, 1987; Pruneau and Belichard, 1993). Hence, in the present study, we used a novel instrument, the microphysiometer to measure functional responses to bradykinin receptor agonists in cultured bovine aortic endothelial cells as well as investigating the effects of lipopolysaccharide. The microphysiometer measures cellular metabolism via extracellular acidification (McConnell et al., 1992). Ligand–receptor interactions in isolated cultured cells lead to a functional response that requires energy. Energy production and metabolism by the cells leads, ultimately, to the production and excretion of protons. Consequently, receptor activation will result in an increase in the rate of extracellular acidification and this is the biological basis of the measurements made using the microphysiometer (Fig. 1). The results of our investigation using this instrument show that, in addition to bradykinin B_2 receptors, bradykinin B_1 receptors are constitutively expressed in cultured bovine aortic endothelial cells and that lipopolysaccharide mediates a significant decrease in bradykinin B_1 and B_2 receptor-mediated acidification responses in isolated cultured bovine aortic endothelial cells.

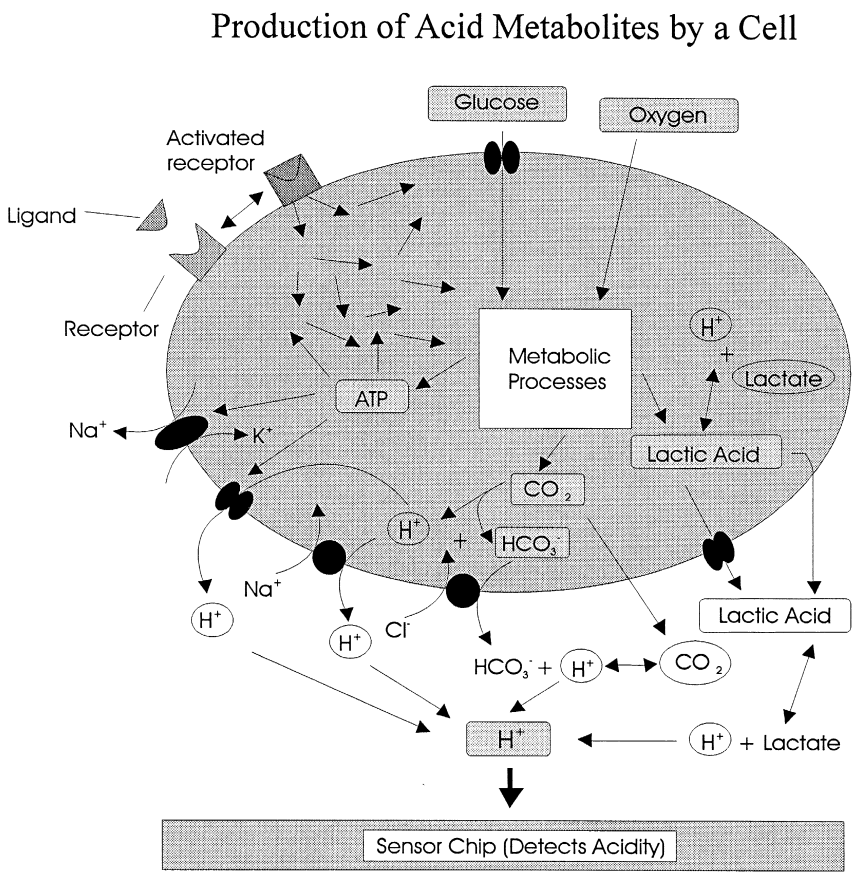

Fig. 1. A schematic diagram which shows the intracellular events that lead to extracellular acidification. The microphysiometer detects the protons released by means of a light-addressable potentiometric sensor (LAPS) (diagram modified from McConnell et al., 1992).

2. Methods

2.1. Cell culture

Bovine aortic endothelial cells were obtained from Dr J Chin (Baker Medical Institute, Melbourne: for method of isolation see Cocks et al., 1985) where they were positively identified as endothelial cells by immunostaining for von Willebrand factor and their lack of staining for smooth muscle α-actin. Cells were grown in RPMI 1640 supplemented with 15% foetal calf serum (FCS), L-glutamine (2 mM) and gentamycin (50 μg/ml). Cultures were incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2 and subcultured every 3 to 4 days when the cells had reached confluency. Experiments were performed on cells of passage 7 through 16 and cells from at least three different primary cultures were used for the study.

2.2. Experimental procedure

Cells harvested from confluent monolayers were seeded into the microphysiometer capsule cups, (1,000,000 in 1 ml cell culture medium, RPMI 1640 + additives (see Cell culture methods)) on the day prior to experimentation and incubated at 37°C in an atmosphere of 95% air/5% CO_2 to attach to the cup overnight. In the preincubation experiments, lipopolysaccharide was added to the culture media at the time the cells were seeded into the capsules at a density of 10^6 cells/capsule (cells seeded in parallel capsule cups for treated and control experiments were harvested from the same plate of cells). On the day of the experiment, the cell capsules were transferred to the sensor chambers of the microphysiometer and modified, low-buffered RPMI 1640 medium was pumped across the cells at a rate of 100 μl/min during which time the pH of the extracellular environment was constant. To measure the rate at which cells excreted protons, the pump was halted periodically to measure the rate of accumulation of protons in the chamber which was detected by the silicon sensor. Flow was then resumed to wash out the acid and this pump-on/pump-off cycle was repeated throughout the experiment in a uniform procedure, under computer control, which allowed acidification rates to be measured and compared before and after the addition of bradykinin receptor agonists (bradykinin, a bradykinin B_2 receptor agonist or Lys^0–$desArg^{10}$-bradykinin, a bradykinin B_1 receptor agonist) in the absence or presence of appropriate antagonists (icatibant or FR173657, selective bradykinin B_2 receptor antagonists or $desArg^9$–Leu^8-bradykinin, a selective bradykinin B_1 receptor antagonist). Each concentration of agonist was administered by inclusion in the running medium for 8 min (bradykinin) or 10 min (Lys^0–$desArg^{10}$-bradykinin) with a washout period of 15 min (bradykinin) or 20 min (Lys^0–$desArg^{10}$-bradykinin). Antagonists were perfused for 60 min prior to carrying out an agonist concentration–response curve in the continuing presence of the antagonist. Control and antagonist curves were carried out on the same batch of cells seeded in parallel chambers, with the responses being normalised to account for differences in response due to different numbers of cells in each chamber. All experiments were carried out in the presence of the angiotensin converting enzyme inhibitor, captopril (10 μM) and the neutral endopeptidase inhibitor, phosphoramidon (1 μM), to prevent enzymatic breakdown of the exogenously applied peptide drugs.

2.3. Data analysis

EC_{50} values were obtained by computer-assisted nonlinear curve fitting methods (PRISM®, GraphPad Software, San Diego). The dissociation constant for each antagonist was estimated by the equation: $pK_B = \log (CR - 1) - \log$ [antagonist], where: CR (concentration ratio) = EC_{50} of agonist in the presence of antagonist/EC_{50} of agonist in the absence of antagonist. All results were expressed as the mean ± S.E.M. Statistical significance between groups was analysed using the Student's unpaired *t*-test.

2.4. Drugs

Purified bacterial lipopolysaccharide from *Escherichia coli* serotype 0127:B8 was obtained from Sigma and reconstituted in sterilised tissue culture grade water. FR173657 was a generous gift from Fujisawa Pharmaceutical, Japan. Other compounds and their suppliers were bradykinin (Auspep), icatibant (Hoechst), $desArg^9$–Leu^8-bradykinin acetate and captopril (Sigma), Lys^0–$desArg^{10}$-bradykinin (Phoenix Pharmaceuticals), phosphoramidon (Boehringer Mannheim). All drugs were dissolved in distilled water, with the exception of FR173657, which was dissolved in dimethylsulphoxide (DMSO) at a stock concentration of 1 mM and subsequently diluted in the microphysiometer running media. Cell culture materials were obtained from Gibco.

3. Results

3.1. B_2 bradykinin receptor characterisation

Exposure of bovine aortic endothelial cells to concentrations of bradykinin (0.1–100 nM) produced acidification responses with a pEC_{50} of 8.87 ± 0.20 ($n = 10$). These responses were selectively antagonised by the bradykinin B_2 receptor antagonist icatibant (Hoe-140; apparent pK_B = 9.11 ± 0.21, $n = 5$) (Fig. 2a). The non-peptide bradykinin B_2 receptor antagonist FR173657 (0.3 and 3 nM) produced a concentration-related, insurmountable antagonism of bradykinin-induced acidification responses (Fig. 2b). The maximum response was reduced to 74.6 ±

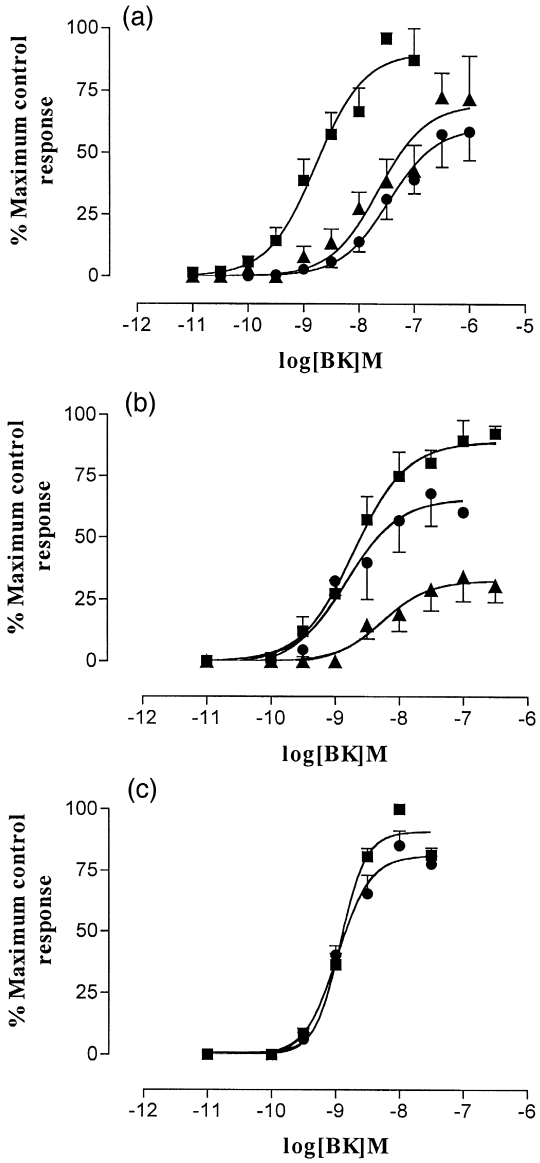

Fig. 2. (a) Concentration–response curves to bradykinin alone (■: $n=10$) and in the presence of 1 (▲: $n=5$) and 3 nM (●: $n=5$) icatibant (Hoe-140), the bradykinin B_2 receptor antagonist. (b) Concentration–response curves to bradykinin alone (■: $n=8$) and in the presence of 0.3 (=: $n=4$) and 3 nM (▲: $n=4$) FR173657, the non-peptide bradykinin (●) B_2 receptor antagonist and (c) concentration–response curves to bradykinin alone (■: $n=4$) and in the presence of 3 μM (●: $n=4$) desArg^9–Leu^8-bradykinin, the bradykinin B_1 receptor antagonist. Acidification responses from bovine aortic endothelial cells were measured using a microphysiometer and expressed as a percentage of the maximum response to bradykinin in the absence of antagonist.

9.2 and 35.4 ± 9.0% (compared to control responses, $n=4$) in the presence of 0.3 and 3 nM FR173657, respectively.

3.2. B_1 bradykinin receptor characterisation

Exposure of bovine aortic endothelial cells to concentrations of Lys^0–desArg^{10}-bradykinin (0.03-3 nM) produced acidification responses with a pEC_{50} of 9.78 ± 0.08 ($n=5$). The maximum response to Lys^0–desArg^{10}-bradykinin was 50.3 ± 5.5% ($n=5$) of the maximum response to bradykinin (after the responses in parallel chambers were normalised to account for differences in responses being

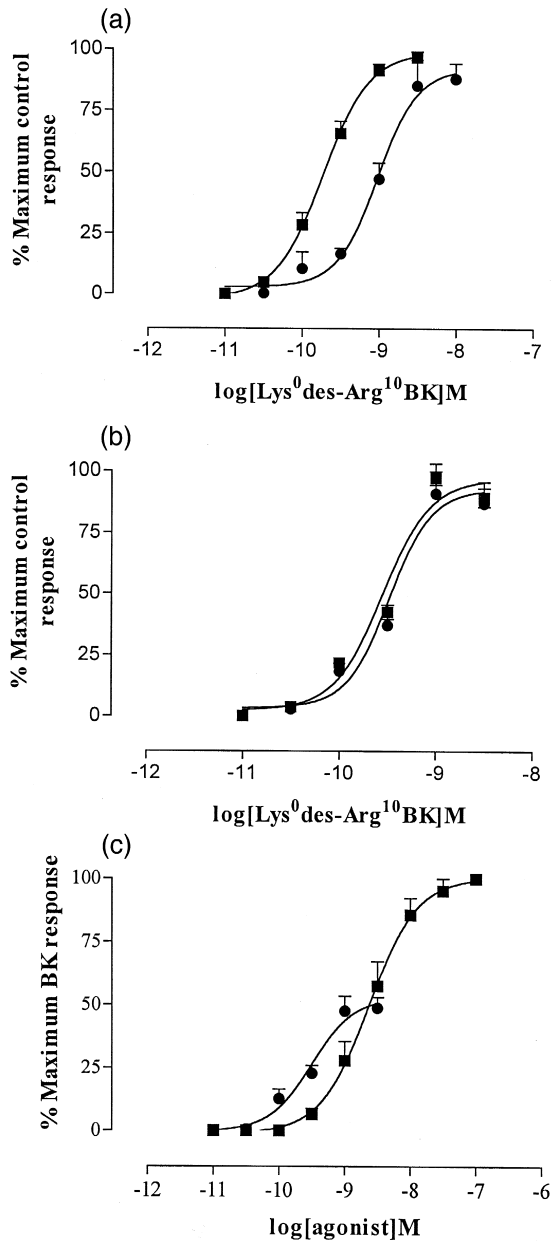

Fig. 3. (a) Concentration–response curves to Lys^0–desArg^{10}-bradykinin alone (■: $n=5$) and in the presence of 3 μM (●: $n=5$) desArg^9–Leu^8-bradykinin and (b) concentration curves to Lys^0–desArg^{10}-bradykinin alone (■: $n=4$) and in the presence and 3 nM (●: $n=4$) FR173657. Acidification responses from bovine aortic endothelial cells were measured using a microphysiometer and expressed as a percentage of the maximum response to Lys^0–desArg^{10}-bradykinin in the absence of antagonist. (c) Concentration–response curves to bradykinin (■: $n=5$) and Lys^0–desArg^{10}-bradykinin (●: $n=5$). The responses to Lys^0–desArg^{10}-bradykinin were expressed as a percentage of the maximum response to bradykinin.

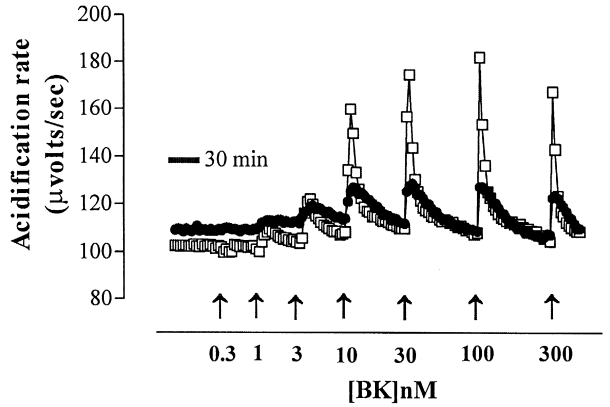

Fig. 4. Treatment of bovine aortic endothelial cells with lipopolysaccharide did not directly affect the basal metabolic rate of these cells measured using a microphysiometer. Original trace showing acidification rate responses in a single experiment in which increasing concentrations of bradykinin were included, for 8 min, in the running media perfusing lipopolysaccharide-untreated (□) and lipopolysaccharide-treated (●: 0.01 μg/ml) cells.

due to differences in the numbers of cells seeded; Fig. 3c). The responses to Lys^0–$desArg^{10}$-bradykinin were competitively and selectively antagonised by the bradykinin B_1 receptor antagonist $desArg^9$–Leu^8-bradykinin with a calculated pK_B of 6.19 ± 0.32, $n = 5$ (Fig. 3a). Conversely, $desArg^9$–Leu^8-bradykinin, at the same concentration (3 μM) which antagonises the response to Lys^0–$desArg^{10}$-bradykinin, did not affect acidification responses to bradykinin ($n = 4$; Fig. 2c). Furthermore, FR173657 at the same concentration (3 nM), which antagonises responses to bradykinin, did not affect acidification responses to Lys^0–$desArg^{10}$-bradykinin ($n = 4$; Fig. 3b).

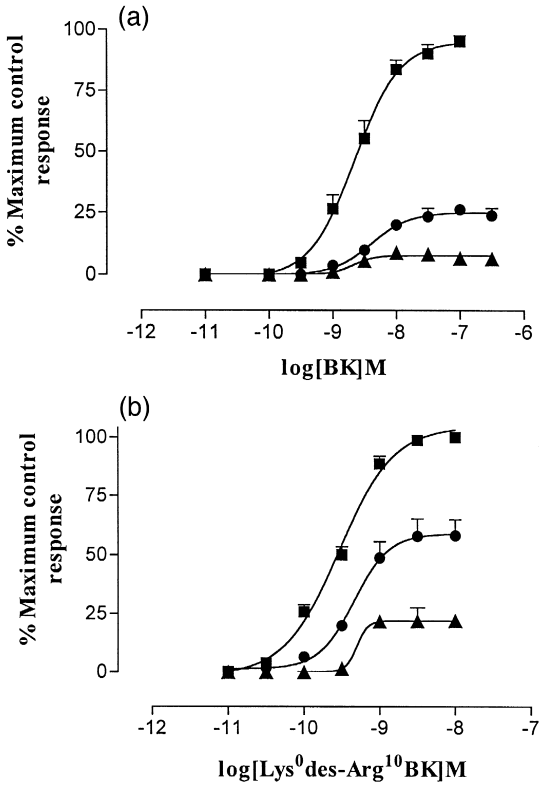

Fig. 5. Bacterial lipopolysaccharide treatment of bovine aortic endothelial cells decreased bradykinin B_1 and B_2 receptor-mediated acidification responses. Cells were seeded in the capsules cups with or without lipopolysaccharide and responses to agonists were measured 24 h later using a microphysiometer. (a) Concentration–response curves to bradykinin in lipopolysaccharide-untreated (■: $n = 8$) and lipopolysaccharide-treated (●: 0.01 μg/ml, and ▲: 0.1 μg/ml, $n = 4$) cells and (b) concentration–response curves to Lys^0–$desArg^{10}$-bradykinin in lipopolysaccharide-untreated (■: $n = 8$) and lipopolysaccharide-treated (●: 0.01 μg/ml and ▲: 0.1 μg/ml, $n = 4$) cells.

Table 1
Effect of lipopolysaccharide (LPS) on pharmacological characteristics of bradykinin B_2 receptors in bovine aortic endothelial cells

	Bradykinin			
	−LPS	+LPS (0.01 μg/ml)	−LPS	+LPS (0.1 μg/ml)
pEC_{50}	8.39 ± 0.07	8.35 ± 0.06	8.72 ± 0.09	8.51 ± 0.15
R_{max}	100	27.2 ± 1.9[a]	100	9.7 ± 0.4[a]

Data represents the mean ± S.E.M., $n = 4$.
[a] $P < 0.01$, significantly different to the corresponding value in the absence of lipopolysaccharide (Student's unpaired *t*-test).

3.3. Effect of bacterial lipopolysaccharide on Bradykinin B_1 and B_2 bradykinin receptor-induced acidification responses

The effect of bacterial lipopolysaccharide (0.01 μg/ml) on the acidification responses to bradykinin is shown in Fig. 4. A 24-h preincubation of bovine aortic endothelial cells with lipopolysaccharide caused a significant concentration-dependent decrease in the maximal response to bradykinin (R_{max}, 27.2 ± 1.9 and 9.7 ± 0.4% of control for 0.01 and 0.1 μg/ml lipopolysaccharide, respectively, $n = 4$; Fig. 5a) without affecting the pEC_{50} (see Table 1). Similarly, an identical treatment of bovine aortic endothelial cells with lipopolysaccharide caused a significant decrease in the maximal response to Lys^0–$desArg^{10}$-bradykinin (R_{max}, 59.0 ± 7.14 and 25.3 ± 7.8% of control for 0.01 and 0.1 μg/ml lipopolysaccharide, respectively, $n = 4$; Fig. 5b) without affecting the pEC_{50} (see Table 2). Lipopolysaccharide had no direct effect on the metabolic

Table 2
Effect of lipopolysaccharide (LPS) on pharmacological characteristics of bradykinin B_1 receptors in bovine aortic endothelial cells

	Lys^0–$desArg^{10}$-bradykinin			
	−LPS	+LPS (0.01 μg/ml)	−LPS	+LPS (0.1 μg/ml)
pEC_{50}	9.44 ± 0.02	9.34 ± 0.53	9.62 ± 0.07	9.32 ± 0.09
R_{max}	100	59.0 ± 7.1[a]	100	25.3 ± 7.8[b]

Data represents the mean ± S.E.M., $n = 4$.
[a] $P < 0.05$, significantly different to the corresponding value in the absence of lipopolysaccharide (Student's unpaired *t*-test).
[b] $P < 0.01$, significantly different to the corresponding value in the absence of lipopolysaccharide (Student's unpaired *t*-test).

integrity of the endothelial cells as indicated by the comparable basal metabolic rate measured in control and treated cells (Fig. 4).

4. Discussion

In this study, we have characterised both bradykinin B_2 and B_1 receptors on endothelial cells from the bovine aorta, using a microphysiometer. We have demonstrated that the microphysiometer is a robust technique to characterise G-protein linked receptors. Thus, the average maximum acidification response measured in the present study was five times greater than that reported in a recent study in which the microphysiometer was used to functionally characterise an ion-channel linked receptor, the $GABA_A$ receptor, in rat cultured cerebellar granule cells (Brown et al., 1997). In the present study, acidification responses to bradykinin were antagonised by icatibant (Hoe-140) and FR173657 while those to Lys^0–$desArg^{10}$-bradykinin were antagonised by $desArg^9$–Leu^8-bradykinin but not vice versa. Bradykinin caused a far greater maximum extracellular acidification than Lys^0–$desArg^{10}$-bradykinin. This could be a direct reflection of the number of each subtype of receptor on each cell or else a reflection of the fact that the activation of bradykinin B_1 receptors and their effector systems requires less energy than bradykinin B_2 receptor activation. Furthermore, we demonstrated that bacterial lipopolysaccharide reduced the acidification responses to both bradykinin and Lys^0–$desArg^{10}$-bradykinin in a concentration-dependent manner, reducing the maximum response (R_{max}) without affecting the EC_{50}. Lipopolysaccharide appears to be more efficient against bradykinin than Lys^0–$desArg^{10}$-bradykinin-induced acidification responses presumably because the effector that is being affected by lipopolysaccharide contributes a larger proportion of the overall acidification response to bradykinin than to Lys^0–$desArg^{10}$-bradykinin. In support of this, a study using bovine pulmonary artery endothelial cells has shown that [^{3}H]inositol triphosphate production induced by $desArg^9$-bradykinin, a bradykinin B_1 receptor agonist, was about half that evoked by bradykinin even though they were equally efficacious in increasing intracellular calcium (Smith et al., 1994). It is possible that lipopolysaccharide could be affecting the same pathway in bovine aortic endothelial cells which ultimately causes the release of nitric oxide. This lipopolysaccharide-induced reduction in receptor-mediated acidification response was not a consequence of reduced cell viability since the basal metabolic rate measured between control and treated cells was comparable.

These data are in contrast to previous studies using both in vitro tissue techniques and in vivo animal models in which it has been shown that endotoxin (lipopolysaccharide) or cytokines lead to the induction of bradykinin B_1 receptor-mediated responses. For example, in a thermal hyperalgesia rat model (Perkins et al., 1995) and a mechanical hyperalgesia rat model (Davis and Perkins, 1994), injection of interleukin-1β induced hyperalgesia mediated by $desArg^9$-bradykinin, a bradykinin B_1 receptor agonist. Other studies reported that $desArg^9$-bradykinin induced hypotension in rabbits, but only after an injection of lipopolysaccharide (e.g. Drapeau et al., 1991) and Deblois et al. (1988) reported that both interleukin-1 and interleukin-2 potentiated the spontaneously developing contractile response to $desArg^9$-bradykinin in rabbit isolated aorta. While our data differ from previous reports (DeBlois et al., 1988; Drapeau et al., 1991; Davis and Perkins, 1994; Perkins et al., 1995), it should be noted that with respect to vascular tissue, all previous studies reporting that lipopolysaccharide or cytokines induce/increase bradykinin receptor-mediated effects have been in vivo or in intact tissue in vitro and, hence, the overall response is generated by a heterogeneous population of cells.

The obvious difference between the latter studies and the present one is that an isolated homogenous population of endothelial cells was used in the present study to discern the effect of lipopolysaccharide in this cell type without the confounding effects of other different cell types. An evaluation of the effect of lipopolysaccharide on heterogenous vascular smooth muscle cell/endothelial cell cultures may possibly reveal a different effect of lipopolysaccharide. Indeed, it has been shown that vascular smooth muscle cells can interact with endothelial cells to produce an overall different response to that which is seen when endothelial cells are cultured alone. For example, Bonin and Damon (1994) reported that the amount of endothelin-1 secreted into the medium was markedly reduced in endothelial cell/vascular smooth muscle cell co-cultures than in homotypic cultures. Furthermore, Loeb et al. (1987) reported that exposure of endothelial cells or vascular smooth muscle cells to endothelium-dependent vasodilators did not produce significant levels of intracellular cGMP. However, when the same vasodilators were applied to endothelial cells and vascular smooth muscle cells in co-culture, significant increases in intracellular cGMP were detected. Similar to the present study, Graier et al. (1994) used homotypic cultures to evaluate the direct effects of bacterial endotoxin (lipopolysaccharide) on agonist stimulated cytosolic Ca^{2+} mobilisation and NO biosynthesis in bovine and porcine endothelial cells. They demonstrated that endotoxin directly decreased the agonist (bradykinin and ADP)-mediated biosynthesis and release of NO from endothelial cells. Considering the plethora of evidence which suggests an induction/upregulation of bradykinin receptors by lipopolysaccharide, the decrease in bradykinin receptor-mediated responses detected in the present study was initially unexpected. Our results, however, like those of Graier et al. (1994) may indirectly support the idea that vascular smooth muscle cells interact with endothelial cells to mediate the overall vascular response. Further studies will involve the use of co-culture system to investigate the

effects of lipopolysaccharide on vascular smooth muscle cell/endothelial cell function.

We have reported in the present study that FR173657 inhibited acidification responses to bradykinin, the bradykinin B_2 receptor-preferring agonist, without affecting the acidification responses to the bradykinin B_1 receptor-selective agonist Lys^0–$desArg^{10}$-bradykinin. To our knowledge, we are the first to characterise this novel orally active non-peptide bradykinin B_2 receptor antagonist on bradykinin receptors expressed by isolated cultured bovine aortic endothelial cells. In our hands, this compound proved to be a potent, insurmountable antagonist, a common property associated with this compound in numerous in vitro assays, although some reports are conflicting. In the first study to identify FR173657 as a bradykinin B_2 receptor antagonist, Asano et al. (1997) demonstrated that FR173657 displaced [^{3}H]-bradykinin binding to bradykinin B_2 receptors in guinea-pig ileum and rat uterine membranes and human bradykinin B_2 receptors expressed in the human lung fibroblast cell line, IMR-90, in a competitive manner. Similarly, a competitive inhibition of the bradykinin-induced contraction of the guinea-pig ileum was observed. In another investigation, FR173657 reportedly inhibited bradykinin B_2 receptor-mediated relaxation of rat isolated duodenum, contraction of guinea-pig ileum and isolated trachea and vasoconstriction of the rabbit isolated perfused ear in an insurmountable manner, whereas it produced a competitive inhibition of bradykinin-induced contraction of rat isolated uterus (Griesbacher et al., 1997). In a later study, Griesbacher et al. (1998) also showed that bradykinin-induced contractions of rabbit isolated sphincter muscle, mediated by tachykinin release from trigeminal afferent neurons, were inhibited by FR173657 in a non-competitive manner. Similarly, FR173657 inhibited bradykinin B_2 receptor-mediated phosphatidylinositol hydrolysis with a concomitant decrease in maximum response (Aramori et al., 1997). Summarising these latter reports and in agreement with the present study, FR173657 acts primarily as a non-competitive bradykinin B_2 receptor antagonist, with conflicting reports relating to its effects at guinea-pig ileum where it has been described as both a competitive and non-competitive antagonist. These conflicting reports may be due to different concentrations of DMSO used as a solvent for FR173657; however, recent data from our laboratory indicates that DMSO has no effect in our system.

The results of the present study also indicate that bradykinin B_1 receptors are constitutively expressed in bovine aortic endothelial cells, which was not entirely unexpected since bradykinin B_1 receptors are known to be induced under conditions in which the vascular tissue has been traumatised. The process of simply mounting a segment of artery into an organ bath will result in the induction of bradykinin B_1 receptor-mediated responses from an initial null level, in a time-dependent manner (Bouthillier et al., 1987; Deblois and Marceau, 1987; Levesque et al., 1995). It would, therefore, be rather surprising if the isolation of bovine aortic endothelial cells by enzymatic dissociation did not result in the apparent 'constitutive' expression of bradykinin B_1 receptors in these cells. Previous studies have reported the presence of B_1 bradykinin receptors on primary bovine aortic endothelial cells before or at the first passage (D'Orleans-Juste et al., 1989; Wiemer and Wirth, 1992), precluding the need, in the present study, to investigate the existence or appearance of bradykinin B_1 receptors on new isolates or early subcultures of bovine aortic endothelial cells.

In conclusion, we have demonstrated, using a microphysiometer, that bradykinin B_1 as well as bradykinin B_2 receptors are constitutively expressed in isolated cultured bovine aortic endothelial cells. In addition, bacterial lipopolysaccharide had an inhibitory effect on acidification responses mediated by both bradykinin B_1 and B_2 receptor subtypes in this homogenous cell system, which contrasts with data obtained on mixed cell populations.

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References

Aramori, I., Zenkoh, J., Morikawa, N., O'Donnell, N., Asano, M., Nakamura, K., Iwami, M., Kojo, H., Notsu, Y., 1997. Novel subtype-selective nonpeptide bradykinin receptor antagonists FR167344 and FR173657. Mol. Pharmacol. 51, 171–176.

Asano, M., Inamura, N., Hatori, C., Sawai, H., Fufiwara, T., Katayama, A., Kayakiri, S., Abe, Y., Inoue, T., Sawada, T., Nakahara, Y., Oku, K., Okuhara, T., 1997. The identification of the orally active, nonpeptide bradykinin B_2 receptor antagonist, FR173657. Br. J. Pharmacol. 120, 617–624.

Barabé, J., Babiuk, C., Regoli, D., 1982. Binding of [^{3}H]des-Arg^9-BK to rabbit anterior mesenteric vein. Can. J. Physiol. Pharmacol. 60 (12), 1551–1555.

Beutler, B., Cerami, A., 1986. Cachetin and tumour necrosis factor as two sides of the same biological coin. Nature 320, 584–588.

Bhoola, K.D., Figueroa, C.D., Worthy, K., 1992. Bioregulation of kinins: kallikreins, kininogens and kininases. Pharmacol. Rev. 44, 1–80.

Bonin, L.R., Damon, D.H., 1994. Vascular cell interactions modulate the expression of endothelin-1 and platelet-derived growth factor BB. Am. J. Physiol. 267, H1698–H1706.

Bouthillier, J., Deblois, D., Marceau, F., 1987. Studies on the induction of pharmacological responses to des-Arg^9-bradykinin in vitro and in vivo. Br. J. Pharmacol. 92, 257–264.

Brown, M.J., Wood, M.D., Coldwell, M.C., Bristow, D.R., 1997. Measurement of $GABA_A$ receptor function in rat cultured cerebellar granule cells by the Cytosensor Microphysiometer. Br. J. Pharmacol. 121, 71–76.

Cocks, T.M., Angus, J.A., Campbell, J.H., Campbell, G.R., 1985. Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture. J. Cell Physiol. 123, 310–320.

Davis, A.J., Perkins, M.N., 1994. The involvement of bradykinin B_1 and B_2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat. Br. J. Pharmacol. 113, 63–68.

Deblois, D., Marceau, F., 1987. The ability of des-Arg^9-BK to relax rabbit mesenteric arteries is acquired during in vitro incubation. Eur. J. Pharmacol. 142, 141–144.

Deblois, D., Bouthillier, J., Marceau, F., 1988. Effect of glucocorticoids, monokines and growth factors on the spontaneous responses of the rabbit isolated aorta to desArg^{9}BK. Br. J. Pharmacol. 93, 969–977.

Deblois, D., Bouthillier, J., Marceau, F., 1991. Pulse exposure to protein synthesis inhibitors enhances vascular responses to des-Arg^9-bradykinin: possible roles of interleukin-1. Br. J. Pharmacol. 103, 1057–1066.

D'orleans-Juste, P., De Nucci, G., Vane, J.R., 1989. Kinins act on B_1 or B_2 receptors to release conjointly endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells. Br. J. Pharmacol. 96, 920–926.

Drapeau, G., Deblois, D., Marceau, F., 1991. Hypotensive effects of Lys–des-Arg^9 bradykinin and metabolically protected agonists of B_1 receptors for kinins. J. Pharmacol. Exp. Ther. 259, 997–1003.

Dray, A., Perkins, M., 1993. Bradykinin and inflammatory pain. Trends Neurosci. 16, 99–104.

Galizzi, J.P., Bodinier, M.C., Chapelain, B., Ly, S.M., Coussy, L., Giraud, S., Neliat, G., Jean, T., 1994. Up-regulation of [^{3}H]-des-Arg^{10}-kallidin binding to the bradykinin B_1 receptor by interleukin-1β in isolated smooth muscle cells: correlation with B_1 agonist-induced PGI_2 production. Br. J. Pharmacol. 113, 389–394.

Graier, W.F., Myers, P.R., Rubin, L.J., Adams, R., Parker, J.L., 1994. *Escherichia coli* endotoxin inhibits agonist-mediated cytosolic Ca^{2+} mobilization and nitric oxide biosynthesis in cultured endothelial cells. Circ. Res. 75, 659–668.

Griesbacher, T., Sametz, W., Legat, F.J., Diehart, S., Hammer, S., Juan, H., 1997. Effects of the non-peptide B_2 antagonist FR173657 on kinin-induced smooth muscle contraction and relaxation, vasoconstriction and prostaglandin release. Br. J. Pharmacol. 121, 469–476.

Griesbacher, T., Sametz, W., Legat, F.J., Diehart, S., Hammer, S., Juan, H., 1998. The nonpeptide B_2 receptor antagonist FR173657: inhibition of effects of bradykinin related to its role in nociception. Br. J. Pharmacol. 124, 1328–1334.

Hall, J.M., 1997. Bradykinin receptors. Gen. Pharmacol. 28, 1–6.

Hock, F.J., Wirth, K., Albus, U., Linz, W., Gerhards, H.J., Wiemer, G., Breipohl, G., Konig, W., Knolle, J., Scholkens, B.A., 1991. Hoe 140, a new potent and long acting bradykinin-antagonist: in vitro studies. Br. J. Pharmacol. 102, 769–773.

Levesque, L., Larrivee, J.F., Bachvarov, D.R., Rioux, F., Drapeau, G., Marceau, F., 1995. Regulation of kinin-induced contraction and DNA synthesis by inflammatory cytokines in the smooth muscle of the rabbit aorta. Br. J. Pharmacol. 116, 1673–1679.

Loeb, A.L., Lohns, R.A., Milner, P., Peach, M.J., 1987. Endothelium-derived relaxing factor in cultured cells. Hypertension 9, III-186–III-192.

Marceau, F., Lussier, A., St-Pierre, S., 1984. Selective induction of cardiovascular responses to des-Arg^9-bradykinin by bacterial endotoxin. Pharmacol. 29, 70–74.

McConnell, H.M., Owicki, J.C., Parce, J.W., Miller, D.L., Baxter, G.T., Wada, H.G., Pitchford, S., 1992. The Cytosensor Microphysiometer: biological applications of silicon technology. Science 257, 1906–1912.

Perkins, M.N., Kelly, D., Davis, A.J., 1995. Bradykinin B_1 and B_2 receptor mechanisms and cytokine-induced hyperalgesia in the rat. Can. J. Physiol. Pharmacol. 73, 832–836.

Pruneau, D., Belichard, P., 1993. Induction of bradykinin B_1 receptor-mediated relaxation in the isolated rabbit carotid artery. Eur. J. Pharmacol. 239, 63–67.

Regoli, D., Barabé, J., 1980. Pharmacology of bradykinin and related kinins. Pharmacol. Rev. 32, 1–45.

Regoli, D., Barabé, J., Park, W.K., 1977. Receptors for bradykinin in rabbit aortae. Can. J. Physiol. Pharmacol. 55, 855–867.

Regoli, D., Rhaleb, N.-E., Dion, S., Drapeau, G., 1990. New selective bradykinin receptor antagonists and bradykinin B_2 receptor characterisation. Trends Pharmacol. Sci. 11, 156–161.

Smith, J.A.M., Webb, C., Holford, J., Burgess, G.M., 1994. Signal transduction pathways for B_1 and B_2 bradykinin receptors in bovine pulmonary artery endothelial cells. Mol. Pharmacol. 47, 525–534.

Wiemer, G., Wirth, K., 1992. Production of cyclic GMP via activation of B_1 and B_2 kinin receptors in cultured bovine aortic endothelial cells. J. Pharmacol. Exp. Ther. 262, 729–733.